IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: **TRINH *et al.***	Confirmation No.: 4261
Appl. No.: **09/306,986**	Art Unit: 1652
Filing date: **May 7, 1999**	Examiner: Hutson, R.G.
For: **A Method for Synthesizing a Nucleic Acid Molecule Using a Ribonuclease**	Atty. Docket: IVGN 202

Reply Brief under C.F.R. § 41.41

Mail Stop Appeal Brief - Patents
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Examiner's Answer mailed on February 21, 2007, Appellants file herewith a Reply Brief in accordance with 37 C.F.R. § 41.41, along with a Request for Oral Hearing (USPTO Form SB/32).

It is not believed that extra fees are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional fees are due or any of the fees set forth in 37 C.F.R. § 41.20 have been increased since they were previously paid and are necessary to prevent abandonment of this application, then such fees required are hereby authorized to be charged to our Deposit Account No. 50-3994.

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I. Status of Claims

Claims 8-13, 56, and 70-75 are pending in the application.

Claims 1-7, 14-55, and 57-69 have been canceled.

Claims 8-12, 56 and 70-73 are rejected.

Claims 13, 74 and 75 are objected to.

II. Grounds of Rejection to be Reviewed on Appeal

There are two grounds of rejection to be reviewed on appeal:

Ground 1: Claims 8-12, 56 and 70-73 were rejected under 35 U.S.C. § 102(b) as being anticipated by Major, *Biotechniques 12*:40-43 (1992) (Exhibit 1), as evidenced by Deana and Belasco, *Mol. Microbiol. 51*:1205-1217 (2004).

Ground 2: Claims 8-12, 56, 70, 71, and 73 stand rejected under 35 U.S.C. § 103(a), as being unpatentable over Major, *Biotechniques 12*:40-43 (1992) (Exhibit 1) and Maudru *et al., J. Virological Methods 66*:247-261 (1997). Appellants have traversed this rejection.

III. Argument

A. Ground 1: Anticipation

The Major reference does not anticipate Appellants' claims 8-12, 56 and 70-73. This is because Major does not disclose mixing a preparation containing RNA and double-stranded DNA with a DNA polymerase and a peptide or polypeptide having ribonuclease activity, as specified by Appellants' claims. What Major does disclose is mixing a clarified bacterial lysate that contains RNA, double-stranded DNA, and perhaps a peptide or

polypeptide having ribonuclease activity, with a DNA polymerase. In simpler terms, Major discloses mixing:

> [a preparation containing RNA, double-stranded DNA, and perhaps a peptide or polypeptide having ribonuclease activity]
>
> *with*
>
> [a DNA polymerase].

This is in clear contrast to Appellants' claims, which require mixing:

> [a preparation containing RNA and double-stranded DNA]
>
> *with*
>
> [a DNA polymerase and a peptide or polypeptide having ribonuclease activity].

For this reason, the Examiner's argument that "the mere addition of the Taq DNA polymerase to the clarified bacterial lysate containing RNA, double-stranded DNA and RNase as taught by Major constitutes 'mixing the preparation' with one or more DNA polymerases and RNases" is not correct. See Examiner's Answer at page 11. The Examiner supports this argument, stating:

> "Nothing in appellants' claimed method excludes that the RNAse cannot inherently be a part of the preparation comprising RNA and double-stranded DNA. There is no method step which requires that an RNAse which is external to the preparation be added to the preparation." Id.

In fact, as described above, Appellants' claimed method clearly requires a mixing step whereby RNase activity is added to the preparation.

B. Ground 2: Obviousness

Claims 8-12, 56, 70, 71 and 73 are not obvious over the Major and Maudru references.

The Major reference discloses a PCR assay for screening point mutations, the basic principle being that PCR amplification should yield products only when the 3'-terminal nucleotide of a primer is matched with a DNA template, and should not do so when the 3'-terminal nucleotide of a primer is mismatched with the template. *See* Major at page 42, left column. Counter to this expectation, Major observed products (*i.e.*, "extra minor bands") in PCR assays using mismatched primers and templates. Major, like other researchers, attributed the unexpected products to PCR amplification of the DNA template, despite the primer / template mismatch. *See* Major at page 42, center column (citing Wu *et al*., *Proc. Natl. Acad. Sci. USA 86*:2757-2760 (1989) and Kwok *et al*., *Nucl. Acids Res. 18*:999-1005 (1990).

The Examiner, however, continues to argue that the "extra minor bands" observed in Major's mismatched primer/template reactions is most likely due to the presence of contaminating RNA. The Examiner contends, because Maudru recognized that Taq DNA polymerase (a PCR assay component) can, under some circumstances, use RNA as a template, skilled artisans would have been motivated to add an RNAse activity in Major's assays to eliminate the problem of contaminating RNA.

The fact is that, at the time the present application was filed, skilled artisans understood that the preparations (*i.e.,* bacterial lysates) used in Major's assay contained many factors other than RNA (*e.g.*, various cellular proteins, salts, lipids, signaling

molecules, metabolites, etc.). One researcher identified factors thought to influence PCR assays like Major's, and RNA was not among those factors identified. See, Charlieu, "Chapter 12, Distinction Between Almost-Identical DNA Sequences by Polymerase Chain Reaction," in PCR Technology Current Innovations, pp. 101-106, Griffin and Griffin Eds., (1994), as described in Appellants' Appeal Brief filed October 24, 2006 at page 15. Another researcher even observed results like Major's using preparations lacking RNA. See Kwok *et al.*, *Nucl. Acids Res. 18*:999-1005 (1990), as described in Appellants' Appeal Brief filed October 24, 2006 at page 14-15, bridging paragraph.

It is manifestly evident from the writings of, at least, Major, Kwok, and Charlieu that skilled artisans at the time the present application was filed did not appreciate that contaminating RNA could be responsible for the unexpected PCR products in Major's assays. Skilled artisans simply did not recognize the problem that RNA could pose, and consequently could not, and did not, contemplate solving that problem by including a ribonuclease activity to degrade RNA, as is presently claimed. The Examiner's quick dismissal of Kwok and Charlieu as "not relevant" does not change this fact.

C. Conclusion

In view of the forgoing discussion, Appellants respectfully submit that the subject matter defined by claims 8-12, 56, 70-73 is novel and non-obvious over the cited art and that the Examiner has not met the burden of establishing a *prima facie* case of anticipation or obviousness. Accordingly, Appellants respectfully request that the Board reverse the

Examiner's rejections of these claims under 35 U.S.C. §§ 102 and 103 and remand this application for issue.

Respectfully submitted,

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IV. Claims Appendix

8. A method for synthesizing a nucleic acid molecule from a preparation comprising RNA and double-stranded DNA, said method comprising:

a) mixing the preparation with one or more DNA polymerases, and one or more peptides or polypeptides having ribonuclease activity, wherein said peptides or polypeptides having ribonuclease activity are capable of degrading single-stranded RNA; and

b) incubating said mixture under conditions sufficient to synthesize a nucleic acid molecule complementary to all or a portion of said double-stranded DNA and under which said peptides or polypeptides having ribonuclease activity degrade said single-stranded RNA.

9. The method according to claim 8, wherein said peptide or polypeptide having ribonuclease activity is selected from the group consisting of: RNase A, RNase T1, RNase S, RNase B, RNase C, RNase T2 and enzymatically active fragments, variants, derivatives or mutants thereof.

10. The method according to claim 8, wherein said mixture further comprises one or more components selected from the group consisting of: a) at least one nucleotide; b) at least one suitable buffer for nucleic acid synthesis; and c) at least one primer.

11. The method according to claim 8, wherein said DNA polymerase is thermostable.

12. The method according to claim 11, wherein said thermostable DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tne DNA polymerase, Tma DNA polymerase, Tth DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, Pyrococcus species GB-D DNA polymerase, Pwo DNA polymerase, Bst DNA polymerase, Bca DNA polymerase, Tfl DNA polymerase and enzymatically active fragments, variants, derivatives or mutants thereof.

13. The method according to claim 10, wherein one or more of said nucleotides are detectably labeled.

56. The method of claim 8, wherein said preparation is from any cell or tissue selected from the group consisting of bacteria; insect; bird; fish; plant; yeast; prokaryote; eukaryote; and mammals.

70. A method according to claim 8, wherein said double-stranded DNA comprises an expression vector.

71. A method according to claim 8, wherein said double-stranded DNA comprises a cloning vector.

72. A method according to claim 8, wherein said double-stranded DNA comprises genomic DNA.

73. A method according to claim 8, wherein said double-stranded DNA comprises a plasmid or a cosmid.

74. A method according to claim 8, wherein said double-stranded DNA comprises viral DNA.

75. A method according to claim 8, wherein said double-stranded DNA comprises phage DNA.